Effectivity of GnRH analogue therapy in the patient with endometriosis

Ph.D. Thesis

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2002

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Contents

Abbreviations

AFS	American Fertility Society
BMI	body mass index
CT	computerised tomography
DIUI	donor artificial intrauterine insemination
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
HIUI	homologous artificial intrauterine insemination
IUI	intrauterine insemination
LH	luteinizing hormone
MRI	magnetic resonance imaging
NK	natural killer
OHSS	ovarian hyperstimulation
PID	pelvic inflammatory disease
STD	sexually transmitted disease
TNF	tumour necrosis factor
TUS	transvaginal ultrasonography
WHO	World Health Organization

I. Introduction

Infertility is usually defined, for a given couple, as the inability to achieve pregnancy within a reasonable duration. This time lag, two years in the guidelines provided by the World Health Organization (WHO), is often shorter in clinical practice, depending both on the impatience of the couple and on the celerity of the physician. Such a condition affects between 15% and 20% of the population in most countries, developed and developing. Among these couples, only a small proportion are affected by complete infecundity, i.e. sterility. These couples usually comprise between 3% and 5% of the population of reproductive age. In developed countries, the average fecundability (the "per cycle" probability of conception) generally fluctuates around an average value of 30%. Although the prevalence of unexplained infertility is decreasing, since the clinical and biological diagnostic procedures are becoming more efficient, conditions in which no factor explaining infertility is found in either the man or the woman account for between 5% and 10% of the cases in the varying surveys. Among the female factors, ovulation disorders (whatever their nature) and tubal alterations are the most prevalent conditions. In the great majority, partial or tubal alterations are the consequences of pelvic inflammatory disease following sexually transmitted diseases. Bacterial vaginosis associated with Gardnerella vaginalis, genital mycoplasmas and several anaerobic species, including Mobiluncus is the most prevalent cause of vaginal discharge, another cause of infertility (14). Over the last 30 years, there has been a large increase in the number of infertile patients found to have endometriosis. It is uncertain whether this is an actual increase or is simply a reflection of the more frequent use of laparoscopy in the investigation of the infertile couple.

Endometriosis is a well-known disease in women of reproductive age. The functioning endometrial gland and stroma are situated outside the uterine cavity, and may be accompanied by pains in the small pelvis, infertility, dysmenorrhoea, dyspareunia and adnexal tumours. It is a benign, but usually persistent and progressive disease, second in incidence to myoma. In 95% of the cases, it occurs in women between the menarche and menopause, whereas in the postmenopause a relationship seems to be evident with the oestrogen intake and hormone replacement therapy. Little is known about the real incidence. While an incidence of 20-50% is reported in the course of examinations by laparoscopy in consequence of infertility, an

incidence of 15-25% is mentioned during gynaecological operations performed on account of pain in the small pelvis (13,23,29,32,33,57). Endometriosis of the small pelvis is typically a disease of the age groups of 25-30, while the extragenital form affects mostly the age group 35-40 (31). Although one-third of the patients are free of symptoms, in endometriosis the acute and chronic pain in the small pelvis is the most frequent complaint, which appears during the menstrual cycle and is associated with dysmenorrhoea and dyspareunia. The pain may be constant, on one or both sides, radiating towards the vagina, the groin, the buttocks or the perianal region (44).

In most cases, laparotomy nowadays has replaced by operative laparoscopy, which plays the main role in the primary diagnosis of endometriosis, in the course of which the changes typical of endometriosis become apparent (4,10,16,23,61). In the diagnosis of the disorder, we can directly vizualise the classic endometriotic implant characterized by brown or black pigmentation and fibrosis, while direct biopsy can be performed on the non-characteristic lesions, which facilitate the true diagnosis. Originally straightforward and easy, the diagnosis has developed into an art which requires a complete knowledge of the symptomatology of the various aspects, and above all an in-depth interpretation of all of the peritoneal anomalies. The most frequent sites of occurrence of the changes revealed during surgery are to be seen in Table I.

Locations of endometriosis

Ovaries
Posterior cul-de-sac
Broad ligament
Uterosacral ligament
Rectosigmoid colon
Bladder
Distal ureter

Table I

1. Peritoneal lesions

It should be realized that the aspect of a peritoneal endometriotic and/or superficial ovarian lesion is the result of the combination of the red of the vessels, the black and pale-brown of the intraglandular debris and the haemosiderin pigment (which we observe through a filter), formed by the peritoneum or the fibrosis, which is more or less opaque, depending on its thickness or age (8,9,29,56).

1.1. Black or dark-blue lesions

These are small black or dark-blue cystic lesions from one to several millimetres in diameter. They are covered and surrounded by a fibrous peritoneal reaction of varying thickness; this variation explains the difference in colour and their aspect, which is more or less retroperitoneal. Histologically, their black colour is due to the presence of debris in the lumen of the glands associated with the stroma and contained by the fibrosis.

These black lesions are typical and are considered to be relatively inactive forms of endometriosis. However, it should be pointed out that they may be located anywhere and that they may only represent the tip of the iceberg, as recently shown by the work of the Koninckx team: macroscopically invasive lesions are similar to typical black lesions (43). It is therefore essential that these lesions can be identified, but their thickness needs to be evaluated to allow suitable treatment to be performed.

1.2. White spots

These thick peritoneal zones are white in appearance, due to the fibrosis by which they are formed. These white zones may contain small black dots, which are thinner zones of fibrosis, in which the glands and haemosiderin pigment content may be seen because of its transparency. The white zones have a fuzzy irregular star-shaped contour, as if the fibrosis surrounding the lesions was thinner at the edges. In this fibrosis, there are rarely glandular elements and they appear to be relatively inactive. Most authors consider these zones to be the

scar zones of previously active lesions, which of course may be widespread. The following should also be remembered:

- white spots are a consequence of peritoneal fibrosis and they may therefore be a consequence of other peritoneal aggressions;
- other, redder aspects which are more active are often to be found on the surface of these white zones, as if the lesions were once again becoming active or intraperitoneal, or as if these zones were the site of successive implantations.

1.3. Excrescence

According to Jansen in 1986, these lesions have the aspect, consistency and colour of the endometrium, as viewed by hysteroscopy (29). This tissue is translucent and shiny in appearance, and its colour and consistency are similar to those of the endometrium.

1.4. Red lesions

Red lesions can have the following aspects:

- red cysts or vesicles, due to the presence of many vessels that may be identified through their transparency and to which they owe the description "red flame-like";
- these red lesions have also been described as having the appearance of a polyp or a red bubble, whose contents are very rich in vessels and which explains this colour.

These small cystic lesions may be as small as 200 Å in diameter. They are therefore only just visible and their diagnosis requires close inspection of the peritoneum. Flat red lesions are much less common, but they may be active.

1.5. Brown lesions

Apart from the lesions situated below the whitish peritoneal layers that may be brownish in colour, brown lesions may have several different macroscopic aspects, ranging

from vesicles to quite often very dark-brown cysts on the surface of the peritoneum, the difference in colour from the red lesions probably being due to differences in the composition of the tissues which form them, the relative number of vessels and an increase in the intracystic debris or slightly different lighting conditions, which may contribute to the change in colour. Finally, the flat brown lesions or brownish areas must also be mentioned, of which two types may be distinguished:

- Dark-brown areas whose aspect is very similar to when the thick, viscous contents of certain endometriomas form deposits on the surface of the peritoneum. Moreover, we suppose that some of these brown areas are a consequence of a recent rupture of an endometrioma, which is often later confirmed by questioning of the patient. As differences from the areas caused by recent surgical rupture, the two following elements may be distinguished:
  - these areas obviously cannot be treated by simple lavage, in the same way as deposits due to a recent rupture;
  - careful inspection shows that there are minute velamentous and vascular adhesions on the surface and the immediate surrounding areas of these lesions.
- Light-brown areas or Jansen coffee stains; these areas are therefore lighter in colour and their contour is often geographical. They are also thinner than the others, the glandular elements that they contain appear relatively inactive and they are few in number, and their colouring is explained by the presence of haemosiderin pigment. They are currently considered to be relatively inactive lesions.

Finally, there are red–brown areas which are often much larger, which we have already described and whose higher vascular content has a higher level of activity.

1.6. Light-coloured lesions

These lesions are in fact light-coloured microvesicles, which are more visible in daylight under low lighting conditions. This is why they are often identified only when the level of lighting has been reduced, when a shutter is used, or on the edge of the video screen, where the light shines not directly, but tangentially.

1.7. Peritoneal defects

These aspects were described by Sampson in the 1930s, and were reassigned to endometriosis by Chatman in 1981. The presence of endometriosis had in fact already been noted by DeBrux and Bret in their evaluation of the Allen and Masters syndrome. However, it was Chatman who showed in two successive studies that these peritoneal defects were much more common in patients with endometriosis (11). In fact, the implants are very often flat red lesions or red or light-coloured vesicles, which may be identified on the edge of or even inside the defect. The physiopathological mechanism of this association has not been fully identified: some consider that it is the presence of the defect which helps the endometriotic implant, while many other pathogenic theories suppose that the endometriosis could create them via the trauma induced in the peritoneum.

1.8. Subovarian adhesions

The adhesions in which the histological examination identifies endometrial glands are most often situated in the ovarian fossa, and thus join the ovary to the peritoneum on the rear face of the broad ligament. It should also be pointed out that these lesions are velamentous, vascular, petechial and often finer, more fragile and more uniformly red than post-infection adhesions. Their presence creates two problems:

- the systematic biopsy of the vascular adhesions to make the aetiological diagnosis of the trauma which has induced them, as it is clear that macroscopy alone is insufficient to identify them reliably;
- treating them requires coagulation or excision of these adhesions.

These endometriotic velamentous vascular adhesions may be located in the ovarian fossa.

1.9. Petechial zones

Donnez described petechial zones and hypervascular zones (16). If endometrial glands may be identified in the peritoneal zones where several petechiae are situated, this aspect must

be interpreted with caution, as laparoscopic instruments can induce petechiae on the peritoneum, which is traumatized when it comes into contact with them. It is therefore essential to observe these fine anomalies on the peritoneum at the beginning of the operation, before the laparoscopic instruments have had an opportunity to modify the image obtained. Furthermore, it is also probable that the laparoscopic instruments induce petechiae more easily on inflamed and abnormal peritoneal zones than on perfectly normal zones.

1.10. Hypervascularized zones

These are red zones containing many dilated and sinuous vessels. These images may also be explained by red layers, which raises the possibility of confluent petechiae (25).

2. Ovarian lesions

Several elements of ovarian endometriosis need to be underlined:

- Lesions on the surface of the ovary can have atypical macroscopic appearances, such as peritoneal lesions, brown lesions, red lesions with brownish deposits and adhesions on the surface of the ovary.
- Puncturing the ovary remains a classic act and is absolutely necessary in the laparoscopic work-up for pelvic endometriosis. Puncturing will in fact allow the endometrioma to be diagnosed in average or small ovaries.
- Puncturing will be replaced by ovariolysis in many cases. In fact, many endometriomas are situated below the mesovarium on the anterior face of the ovary and will be discovered during the adhesiolysis of the upper part of the anterior face of the ovary.

Further, the indications for puncturing the ovary may be guided by two signs, apart from the ovary adhering to the posterior face of the broad ligament, as follows:

- the presence of a star-shaped shrinkage zone on the ovary, especially if this zone is situated on the anterior face of the ovary;

- the presence of a brown zone on the surface of the ovary is also a strong indication of the presence of endometriosis.

Physiopathologically, it is as if the adhesions which form the anterior face of the ovary were drawing the anterior face of the ovary into contact with the broad ligament, with this ovarian adhesion preventing or limiting the utero-ovarian ligament from stretching due to the increased weight of the ovary. Finally, it should be remembered that puncturing with a fine needle is not a pathognomic element of the diagnosis. Experience from echographic punctures has provided confirmation that truly cystic lesions may be white as their contents are so thick that they cannot be sucked up through a needle. The following also needs to be stated about endometriotic cysts:

- their contents may be haemorrhagic, very close to or even identical to that of haemorrhagic luteal cysts, and this shows once again the necessity of a cytological and histological evaluation of all cysts which are "functional" or which are supposed to be when they are found during laparoscopy;
- chocolate fluid is very indicative, but not pathognomic, as around one-third of cysts with chocolate fluid contents are in fact luteal cysts, to the extent that Nezhat proposed that endometriotic cysts were a complication or a development of haemorrhagic luteal cysts.

The ovarian endometrioma must always be considered first to be a tumorous pathology of the ovary. Endometriosis is sufficiently common to be associated with a tumorous pathology of different origin, which requires the same exploration as all ovarian pathologies (62). In addition, it is known that degeneration of the endometriotic lesions is not very common. It is not exceptional, however, and also justifies adopting a very strict attitude for the evaluation of endometriomas.

II. Aims of the investigation

1. To determine the incidence of endometriosis due to infertility and examine the efficacy of gonadotropin releasing hormone (GnRH) analogue therapy in the treatment of the disease.
2. To compare the efficacy of two GnRH analogues, nafarelin (Synarel, Syntex) and triptorelin (Decapeptyl-Depot, Ferring), and their side-effects during a 6-month course of therapy and for another 6 months after the cessation of treatment.
3. To compare the efficacy of assisted reproductive technology, intrauterine insemination (IUI) after GnRH analogue therapy in patients with endometriosis and patients who are free of this disease.
4. To create a treatment protocol for infertile patients who have endometriosis.

III. Patients, materials and methods

On admission, a thorough gynaecological examination was performed in all of the patients included in the studies (colposcopy, cytology and bimanual, together with breast examination). Three months prior to the beginning of the trial, the duration of the menstrual cycle was found to be between 24 and 35 days. Each infertile patient complained of dysmenorrhoea or dyspareunia. None of them was aware of any blood-clotting disturbance. The patients were given information on the need for laparoscopy, the administration of the required drugs and their potential adverse effects. They were asked to keep a therapy record, noting any complications, any symptoms or complaints, and details on the drugs taken. In addition, hyperandrogenic symptoms, mood swings and libido, together with any alteration in the frequency of headaches were noted. During the trial, patients in all groups were asked not to take any hormone products. The degree of intensity, frequency and tolerance of hot flushes were recorded on a visual analogue scale, whose data was based on the patients' complaints. The scale ranged from 0, meaning no pain or discomfort, to 10, at which the symptoms are intolerable. Each month we noted the body weight and blood pressure, and evaluated the

small-pelvic pain according to the results of a physical examination. No patient from either group took any steroids, and the medical history indicated that no patient was pregnant or lactating in the 3 months prior to the trial. We performed videolaparoscopy with chromopertubation prior to the start of the therapy. All the laparoscopies were performed under general endotracheal anaesthesia with a 10 mm diameter 30° laparoscope; in some patients, a biopsy was performed for histological confirmation of endometriosis. Where the diagnosis of endometriosis was uncertain during laparoscopy, histological examination of the biopsy sample confirmed the disease or disproved it. When endometriosis was diagnosed laparoscopically and/or histologically, the therapy was initiated in the first menstrual cycle after the operation.

Several classification methods are available for defining the extent and the consequences of the disease, as well as for assessing the efficiency of the surgical or drug therapy. The score system of the American Fertility Society (AFS), modified in 1985, is currently the most widely accepted, and our classification was made on this basis (Table II) (44,48,76). The obtained scores were utilized to divide the patients into four stages (Table III).

The American Fertility Society (AFS) revised classification system

	ENDOMETRIOSIS	< 1 cm	1-3 cm	> 3 cm
Peritoneum	Superficial	1	2	4
	Deep	2	4	6
Right ovary	Superficial	1	2	4
	Deep	4	16	20
Left ovary	Superficial	1	2	4
	Deep	4	16	20
Posterior cul-de-sac obliteration	Partial	4	Complete	40
	ADHESIONS	<1/3	1/3-2/3	>2/3
Right ovary	Filmy	1	2	4
	Dense	4	8	16
Left ovary	Filmy	1	2	4
	Dense	4	8	16
Right tube	Filmy	1	2	4
	Dense	4*	8*	16
Left tube	Filmy	1	2	4
	Dense	4*	8*	16

* If the fimbriated end of the fallopian tube is completely enclosed, the point assignment is changed to 16.

Table II

AFS staging of endometriosis

Stage I	**(minimal)**	**1-5**
Stage II	**(mild)**	**6-15**
Stage III.	**(moderate)**	**16-40**
Stage IV	**(severe)**	**>40**

Table III

III. 1. Nafarelin study

We performed 122 laparoscopies, indicated by infertility, chronic pain in the small pelvis, dyspareunia or ovarian cyst, with endometriosis as the underlying cause in 30 patients (25%). Following laparoscopy, all the 30 patients involved in our study were given GnRH treatment with nafarelin for 6 months, beginning from the first day of the first menstruation after the surgery. The initial dose of the drug was 2 x 200 μg per day intranasally, applied in both nostrils in turn in order to avoid the problems resulting from the possible local irritation. The use of nasal drops was not recommended between 1 hour before and after the administration of the drug because of vasoconstriction and resulting absorption failure. In the event of penetrating bleeding, the daily dose was raised to 800 μg.

At the onset of the treatment, as well as at the end of the first, third, sixth, ninth and twelfth months, we checked the severity of dysmenorrhoea (Table IV), dyspareunia (Table V) and pain in the small pelvis (Table VI), relying upon the patient's complaints and the examination findings showing the extent of pressure sensitivity of the small pelvis (Table VII).

Within 2-4 weeks following the end of the drug therapy, control laparoscopy was carried out to examine the activity and the extent of the disease, taking the AFS classification into consideration. After the examination, we evaluated the changes in dyspareunia, in dysmenorrhoea, in pain in the small pelvis and in pressure activity.

Clinical findings in endometriosis

Patient report 1

Dysmenorrhoea	**absent**	**no discomfort**
	mild	**some loss of work efficiency**
	moderate	**in bed for part of one day, occasional interruption of work**
	severe	**in bed for one or more days, incapacitation**
	not applicable	**amenorrhoea**

Table IV

Clinical findings in endometriosis

Patient report 2

Dyspareunia	**absent**	**no difficulty or pain**
	mild	**tolerated discomfort**
	moderate	**intercourse painful to the point of interruption of intercourse**
	severe	**avoids intercourse because of pain**
	not applicable	**not sexually active, or prefers not to answer**

Table V

Clinical findings in endometriosis

Patient report 3

Pelvic pain	**absent**	**no discomfort**
	mild	**occasional pelvic discomfort**
	moderate	***noticeable discomfort for most of cycle***
	severe	**requires strong analgesics; persistent during cycle or other than during menstruation**

Table VI

Clinical findings in endometriosis

Pelvic examination

Pelvic tenderness	**absent**	**no tenderness**
	mild	**minimal tenderness on palpation**
	moderate	**extensive tenderness on palpation**
	severe	**unable to palpate because of tenderness**
Induration	**absent**	**no induration**
	mild	**uterus freely mobile, induration in the cul-de-sac**
	moderate	**thickened and indurated adnexa and cul-de-sac, restricted uterine mobility**
	severe	**nodular adnexa and cul-de-sac, uterus frequently frozen**

Table VII

III. 2. Nafarelin and triptorelin study

133 patients with, laparoscopically-diagnosed endometriosis were treated with GnRH analogues. The efficacy and side-effects of nafarelin and triptorelin were compared during a clinical study of a 6-month course of treatment with a 6-month follow-up period. At the onset of the treatment, as well as after every month of the treatment cycle and the seventh, ninth and twelfth months, blood was taken to determine the serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and 17β-oestradiol. There were 30 patients in the nafarelin group and 103 in the triptorelin group. In the first group, the patients' ages ranged from 18 to 42 years of age (average age 31 years), while in the second they ranged from 19 to 45 (average 32 years). In the nafarelin group, 2 patients were given danazol, while in the triptorelin group, 11 patients were given progestogen and/or danazol (but more than 6 months prior to the start of the treatment).

The initial dose of nafarelin was 400 μg, which was administered twice daily as an intranasal insufflation in both nostrils in turn. When breakthrough bleeding occurred, the dose of the drug was raised to an amount no greater than 800 μg. The use of nasal drops was not recommended before or after the nasal insufflation so as to avoid the occurrence of local vasoconstriction and a consequential absorption disorder. Each month, a dose of 3.75 mg triptorelin was administered intramuscularly (given in the deep muscle layer).

III. 3. Triptorelin and ovulation induction study

At our Department ovulation induction and homologous arteficial intrauterine insemination (HIUI) were performed, after GnRH analogue therapy, on 33 patients suffering from laparoscopically diagnosed endometriosis. The patients' ages ranged from 25 to 32 years of age (average age 28.3 years), while their body weights ranged from 65 to 80 kg (average 75.6 kg). One patient was given danazol, but this was more than 6 months prior to the start of the treatment. Prelaparoscopic investigations included evaluation of the basal body temperature, and the vaginal discharge for microbiological culture. Blood was taken to determine the serum levels of FSH, LH, prolactin, testosterone and 17β-oestradiol on day 3 of the menstrual cycle, and again on day 21 of the cycle, to assess the progesterone level. The

hormones were measured by a chemiluminescent immunoassay with a DPC Immulite instrument. In the andrological examination of the partner, a spermiogram was taken and bacteriological testing of the seminal fluid and a postcoital test were performed. 3.75 mg triptorelin was administered intramuscularly each month for 6 months. 21 days after the last injection was given, ovulation induction was started according to the monofollicular protocol. 50 IU Puregon (pure FSH) was given daily for 2 days, and then from the third day, 1 ampoule of Humegon (75 IU FSH, 75 IU LH) was administered intramuscularly (Figure 1). On the fifth or sixth day of the stimulation, transvaginal ultrasonographic (TUS) examination was carried out. If the size of the dominant follicle and/or thickness of the endometrium did not reach the required level, the administration of Humegon was continued and the TUS examination was performed daily. If the dominant follicle reached a size of 20 mm and the endometrium was thicker than 9 mm (consisting of 3 layers), 10.000 IU hCG was given for luteinization after the serum level of oestradiol was determined. Wherever there was a danger of ovarian hyperstimulation, luteinization was not carried out (Table VIII). An intrauterine HIUI was performed 36 hours later. For homologous insemination, the concentration of sperm was determined from the homogenized ejaculate. The washing and enrichment of the sperm was carried out via "swim-up" technology. If the concentration reached $40x10^6$ /ml, 3 ml of washing fluid (Spermfit, BIOMEDICAL) was added to 1 ml of ejaculate. If the concentration did not reach $40x10^6$ /ml, 2 ml of washing fluid was added to 1 ml of ejaculate, the mixture was then centrifuged at 400 g for 10 minutes and the supernatant was drawn off. 1 ml of the medium was layered over a precipitate, care being taken not to agitate the precipitate. The material was incubated at room temperature for 45 minutes, and the sperm concentration, motility characteristics, the percentage, and the percentage of the normal forms were then repeatedly evaluated (Table IX). This procedure was carried out after the administration of hCG, but before the HIUI.

Ovulation induction

Monofollicular protocol

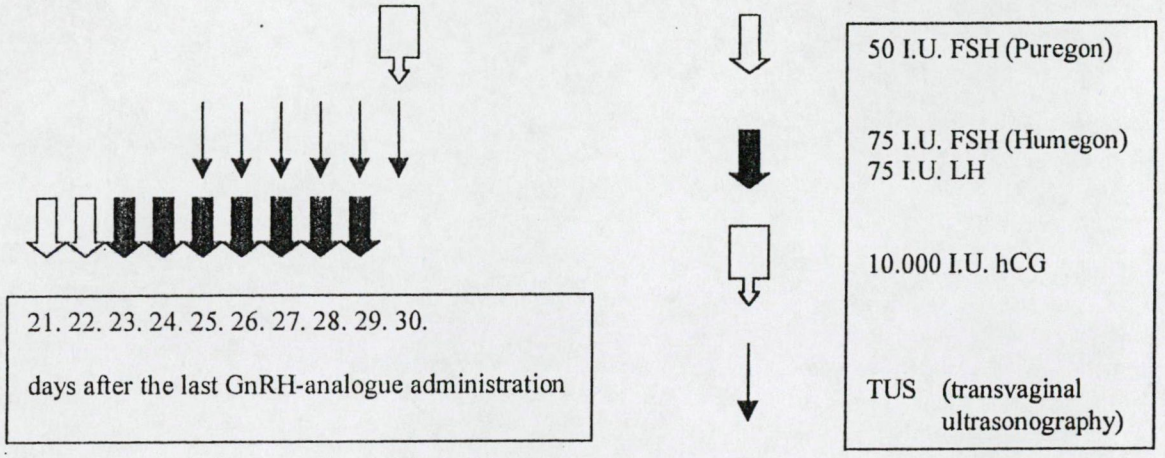

Figure 1

The danger of ovarian hyperstimulation syndrome

DO NOT ADMINISTER hCG!

in the ovary > 10 follicles

10 follicles ≥ 15 mm

ovary size > 10 cm

subjective symptoms (nausea, vomiting)

serum oestradiol level > 6000 pmol/l

Table VIII

Spermium concentration by centrifugation

"Swim-up" technology

1. **Analysis of the sperm**
2. **Addition of wash fluid (Spermfit, Biomedical)**
3. **Centrifugation (for 10 minutes at 400 g)**
4. **Drawing-off the supernatant**
5. **Sperm-concentrate medium**
6. **Incubation**
7. **Analysis of the supernatant:**
   - **spermium concentration**
   - **motility (%)**
   - **motility characteristics**
   - **supravital staining**
   - **morphological examination**

Table IX

III. 4. Ovulation induction and body mass index study

At our Infertility Outpatient Unit, 1144 married couples who attended between 1992 and 1998 requested donor artificial intrauterine insemination (DIUI) treatment. After a thorough history-taking, the infertility examination was carried out on the basis of the criteria given by the WHO: determination of the prolactin and progesterone levels of the serum on days 21-23 of the menstrual cycle, hysterosalpingography and examination of the husbands. We excluded the possibility of endometriosis via the lack of typical symptoms; we did not perform laparoscopy. The Fallopian tubes of the wives were passable, the serum prolactin scores were in the normal range (below 580 mU/l), and their cycles proved to be ovulatory (serum progesterone above 19 nmol/l). Azoospermia or severe oligozoospermia (sperm concentration below 5 million/ml) was found in the husbands. The body mass index (BMI) was determined on the basis of the formula body weight (kg) / body height (m^2). For extremely thin or obese patients, endocrinological examination was required in order to exclude the possibility of endocrine or metabolic diseases. During the preparation for DIUI, superovulatory treatment (clomiphen and gonadotropin) was carried out and the cycles of the wives were monitored. In the course of appropriate follicular maturation, which was proved by ultrasonographic folliculometry and by determination of the serum oestradiol level, 7500 IU hCG was injected to provoke follicular rupture, and 24 hours later the DIUI was performed. The subsequent pregnancy was proved by immune pregnancy testing and TUS.

IV. Results

IV. 1. Nafarelin study

Before the start of the therapy, 20 of the 30 patients were in stage III according to the score system and 10 in stage II. After the treatment, 5 patients were in stage III, 6 in stage II, 7 in stage I and 12 in stage 0 (Figure 2). According to the total AFS score system, which takes into consideration the adhesions, too, recovery was achieved in 11 cases and a partial improvement in 19 cases. Control laparoscopy revealed the total disappearance of the endometrial foci in 21 cases, partial disappearance in 8 cases, no change in 1 case and no aggravation in any of the cases. In the course of the study, a considerable improvement in dysmenorrhoea was found, with entire cessation in 22 cases. The pain in the small pelvis was considerably alleviated, or disappeared following a few aggravations in the beginning. There was a marked improvement or cessation of dyspareunia according to the subjective assessments of patients. From the beginning of the treatment until the end of the second month, there was a discontinuance in menstruation and no bleeding occurred throughout the course of the treatment. Following the cessation of the therapy, the normal menstruation cycle was restored within 56 days in 28 cases. Due to amenorrhoea in 2 cases, bleeding was induced with Norethisterone following day 56. Hot flushes gradually became more intensive from the third week of the treatment; in a few cases headaches, and in 2 cases numbness of the upper limbs with pain were found as side-effects. There was no need to interrupt the study before due time in consequence of side-effects or unexpected complications.

IV. 2. Nafarelin and triptorelin study

No significant differences were observed between the groups in the frequency of hot flushes during the 6-month course of treatment with a 6-month follow-up period (Figures 3 and 4). The hot flushes started in the first month of the analogue therapy, and occurred several times a day; were mild at that time, but as the therapy progressed, the number and intensity increased. The number and intensity of the hot flushes were highest in the third month of the therapy and from the fifth month onwards a decrease in number was noticed. In the first

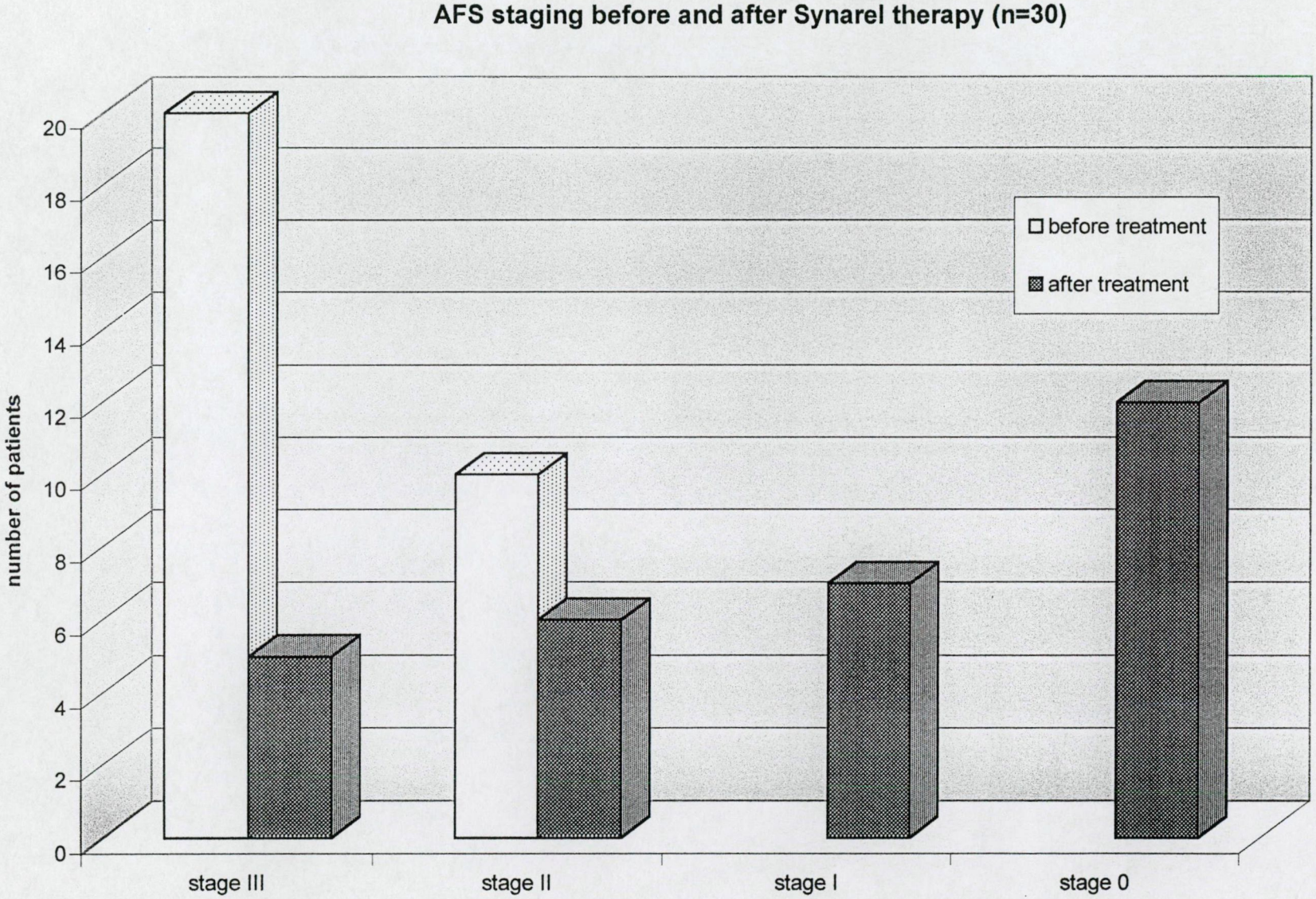
AFS staging before and after Synarel therapy (n=30)
number of patients
0
2
4
6
8
10
12
14
16
18
20
before treatment
after treatment
stage III
stage II
stage I
stage 0

Figure 2

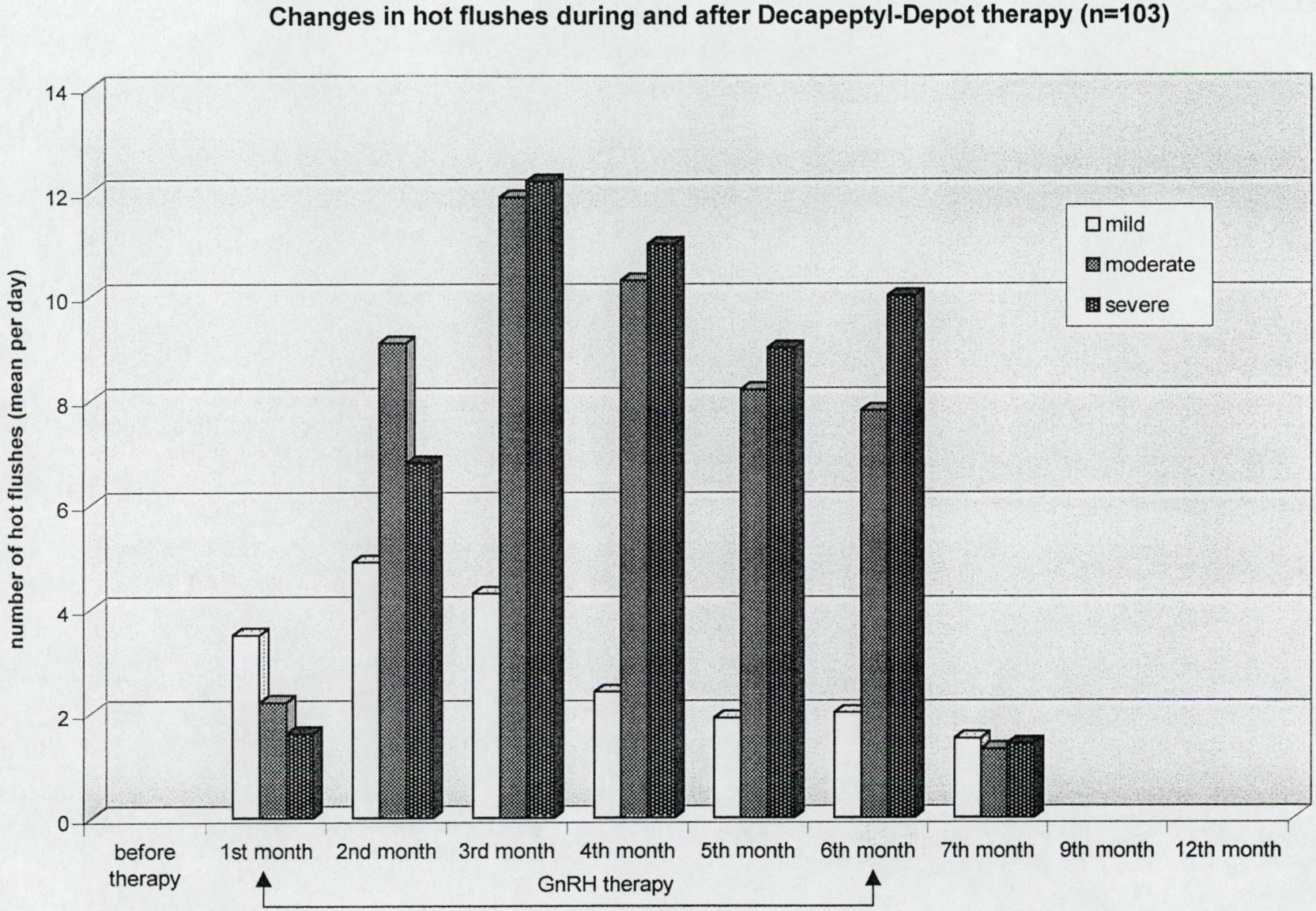

Figure 3

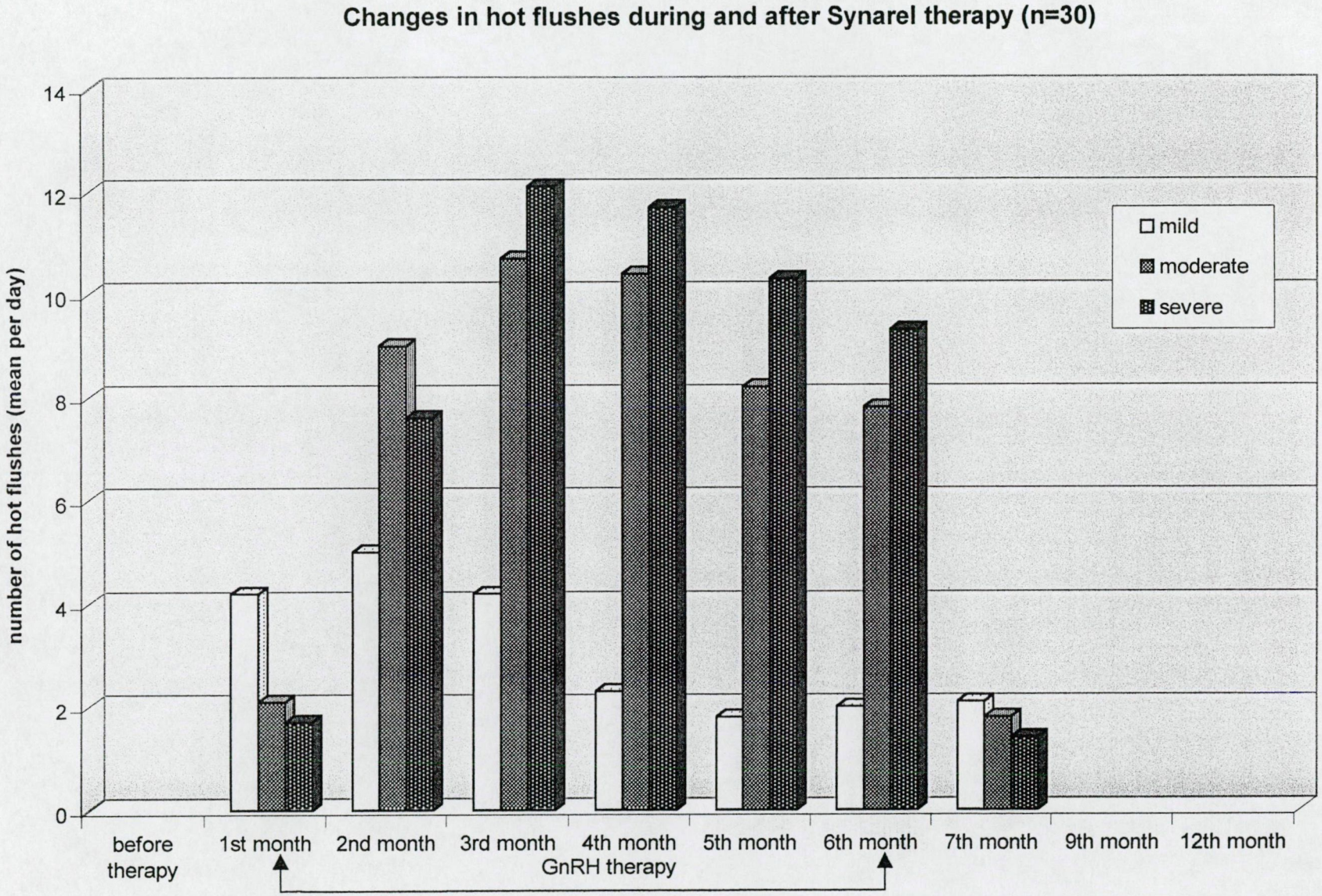
Changes in hot flushes during and after Synarel therapy (n=30)
number of hot flushes (mean per day)
14
12
10
8
6
4
2
0
mild
moderate
severe
before therapy
1st month
2nd month
3rd month
4th month
5th month
6th month
7th month
9th month
12th month
GnRH therapy

Figure 4

month following analogue therapy, reductions were observed in both their number and their intensity. The hot flushes completely disappeared from the second month on following the therapy. In the first month after the start of the medical therapy, the symptoms characteristic of menopause (colpoxerosis, nervousness, mood swings, and reductions in libido and in the size of the breasts, accompanied by hot flushes) appeared due to hypoestrogenism. As regards side-effects no significant difference was found between the two groups (Table X).

Side-effects reported during GnRH analogue therapy (n=133)

	Synarel (n=30)	Decapeptyl-Depot (n=103)
nervousness	40%	38%
mood swings	27%	26%
hyperandrogenic symptoms	11%	10%
decreased libido	35%	30%
headache	13%	10%
decreased breast size	18%	20%
weight gain	6%	5%
weight loss	3%	3%

Table X

There was no significant difference between the serum hormone levels in the two groups during and after the analogue therapy. We found that the GnRH analogue therapy decreased the serum FSH and LH concentrations, and the oestradiol level in all patients was in the postmenopausal range; after the therapy, all the values were within the normal range (Figures 5-7). At the end of the medical therapy, the ovaries were no longer inhibited, so the menopausal symptoms gradually became milder and eventually vanished. After an initial

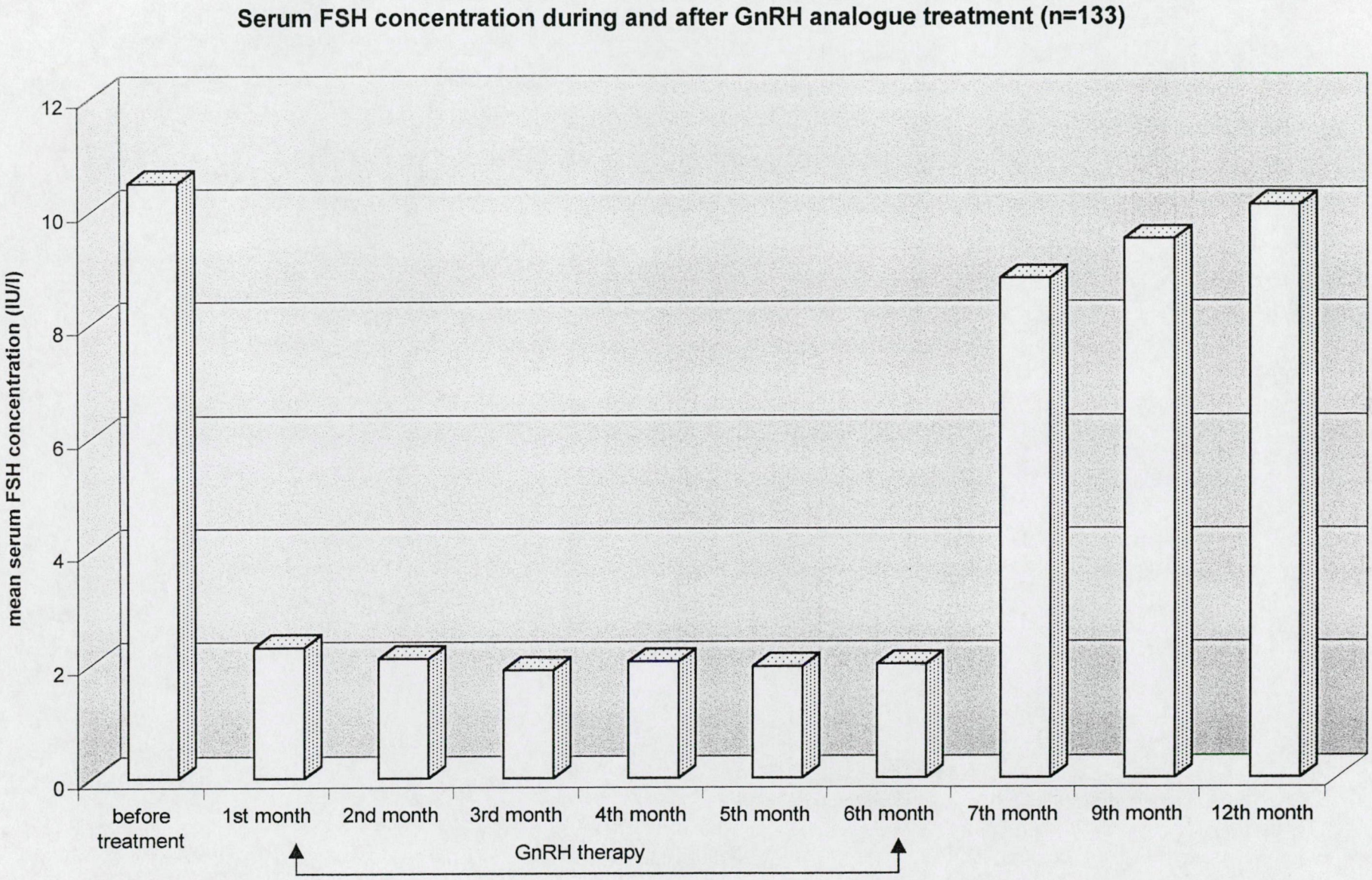
Serum FSH concentration during and after GnRH analogue treatment (n=133)
mean serum FSH concentration (IU/l)
12
10
8
6
4
2
0
before treatment
1st month
2nd month
3rd month
4th month
5th month
6th month
7th month
9th month
12th month
GnRH therapy

Figure 5

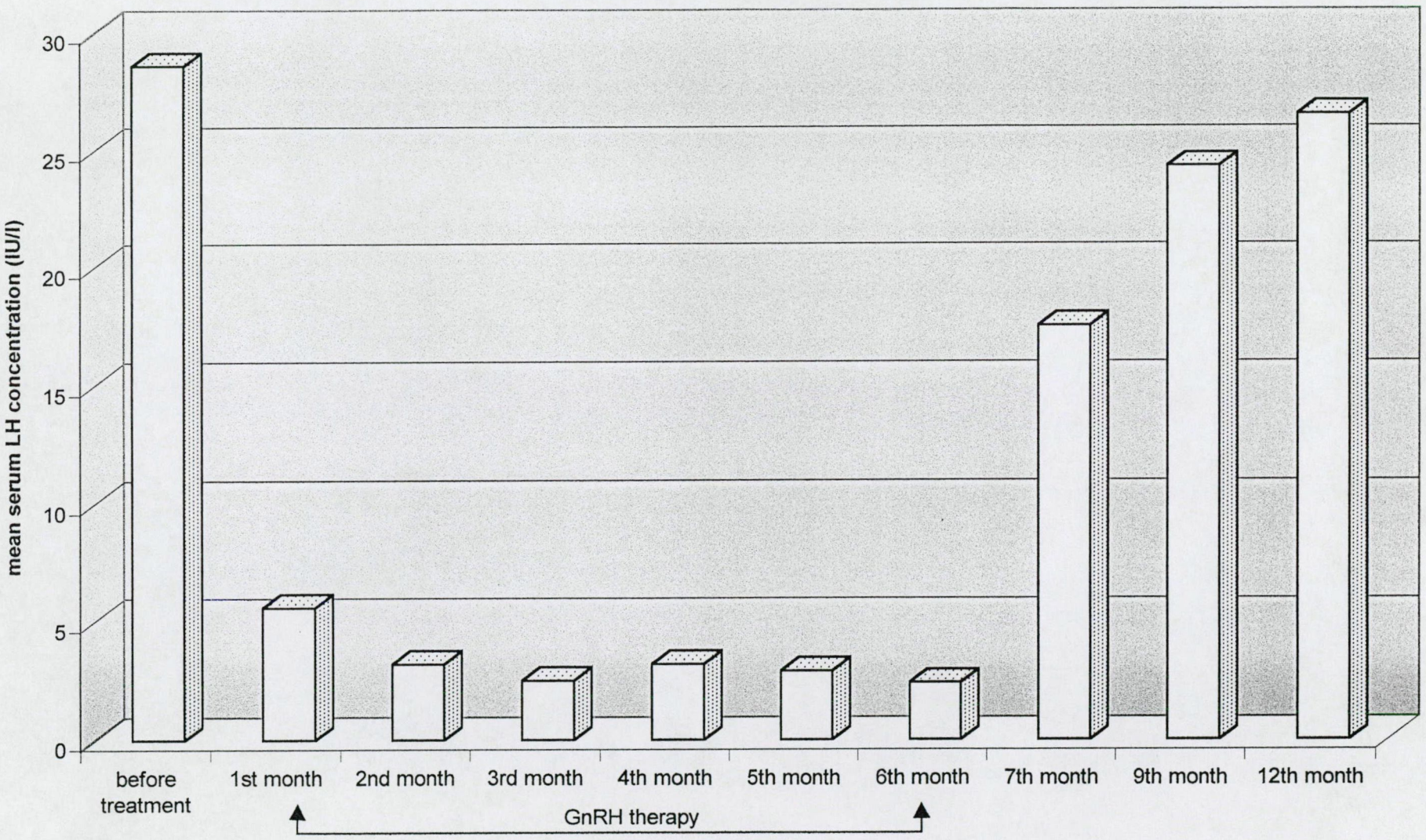
Serum LH concentration during and after GnRH analogue treatment (n=133)
mean serum LH concentration (IU/l)
30
25
20
15
10
5
0
before treatment
1st month
2nd month
3rd month
4th month
5th month
6th month
7th month
9th month
12th month
GnRH therapy

Figure 6

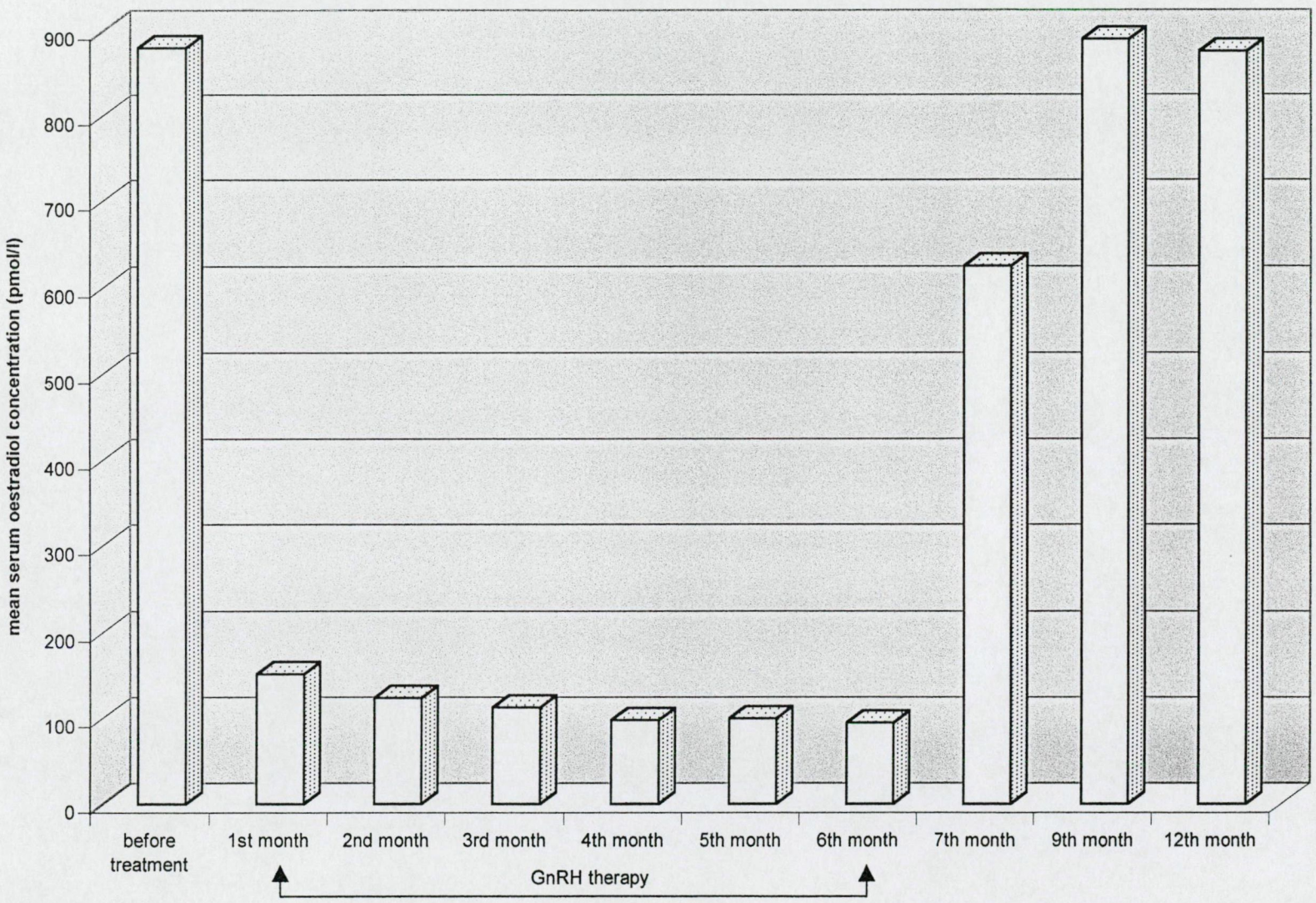
Serum oestradiol concentration during and after GnRH analogue treatment (n=133)
mean serum oestradiol concentration (pmol/l)
900
800
700
600
500
400
300
200
100
0
before treatment
1st month
2nd month
3rd month
4th month
5th month
6th month
7th month
9th month
12th month
GnRH therapy

Figure 7

exacerbation the symptoms characteristic of endometriosis showed an improvement in both groups as compared to those prior to the start of the treatment, which was a consequence of the effect of the analogue therapy. We found no significant complications in either therapy group. When the control laparoscopy was performed later, a significant improvement in the recovery from the disease was inferred. In the nafarelin group, 20 patients were in stage III and 10 in stage II at the beginning of the treatment. However, at the end only 5 patients were in stage III, 6 in stage II, 7 in stage I and the remaining 12 in stage 0 (Figure 2). In the triptorelin group, prior to the start of the treatment 20 patients were in stage IV, 46 in stage III, 31 in stage II and 6 in stage I, but after the treatment only 8 patients were in stage IV, 20 in stage III, 22 in stage II and the remaining 26 in stage 0 (Figure 8). A marked improvement was also seen in those cases where the stage did not change after the treatment, as compared to the stage at the beginning of the treatment.

IV. 3. Triptorelin and ovulation induction study

The serum hormone levels were determined on days 3 and 21 of the menstrual cycle, within 2 months prior to the laparoscopy. In each case, normal hormone levels were detected. The laparoscopy revealed that 10 patients were in stage III, 18 in stage II and the remaining 5 in stage I (Figure 9). Those patients whose tubes were shown to be penetrable via chromopertubation were included in the trial, while those with one or both Fallopian tubes occluded were excluded from the trial. GnRH analogues were naturally prescribed for the treatment of endometriosis. The andrological analysis in each case found normozoospermia. In 4 cases, the bacteriological test detected the presence of a pathogen, so targeted antibiotic therapy was given in accordance with the antibiogram. In 1 case, after the control test, targeted antibiotic therapy was repeatedly given until a negative result was attained.

In the first month of the induction treatment, 5-8 (6.8 on average) ova reached a size of 20 mm up to day 12 of the induction, which is in part due to the rebound effect following the inhibition. The follicle size ranged from 20 to 28 mm (24.4 mm on average). The intervention resulted in pregnancy in 15 (45%) cases, 3 of them being twin pregnancies. In 1 case, a spontaneous abortion took place in week 15 of pregnancy.

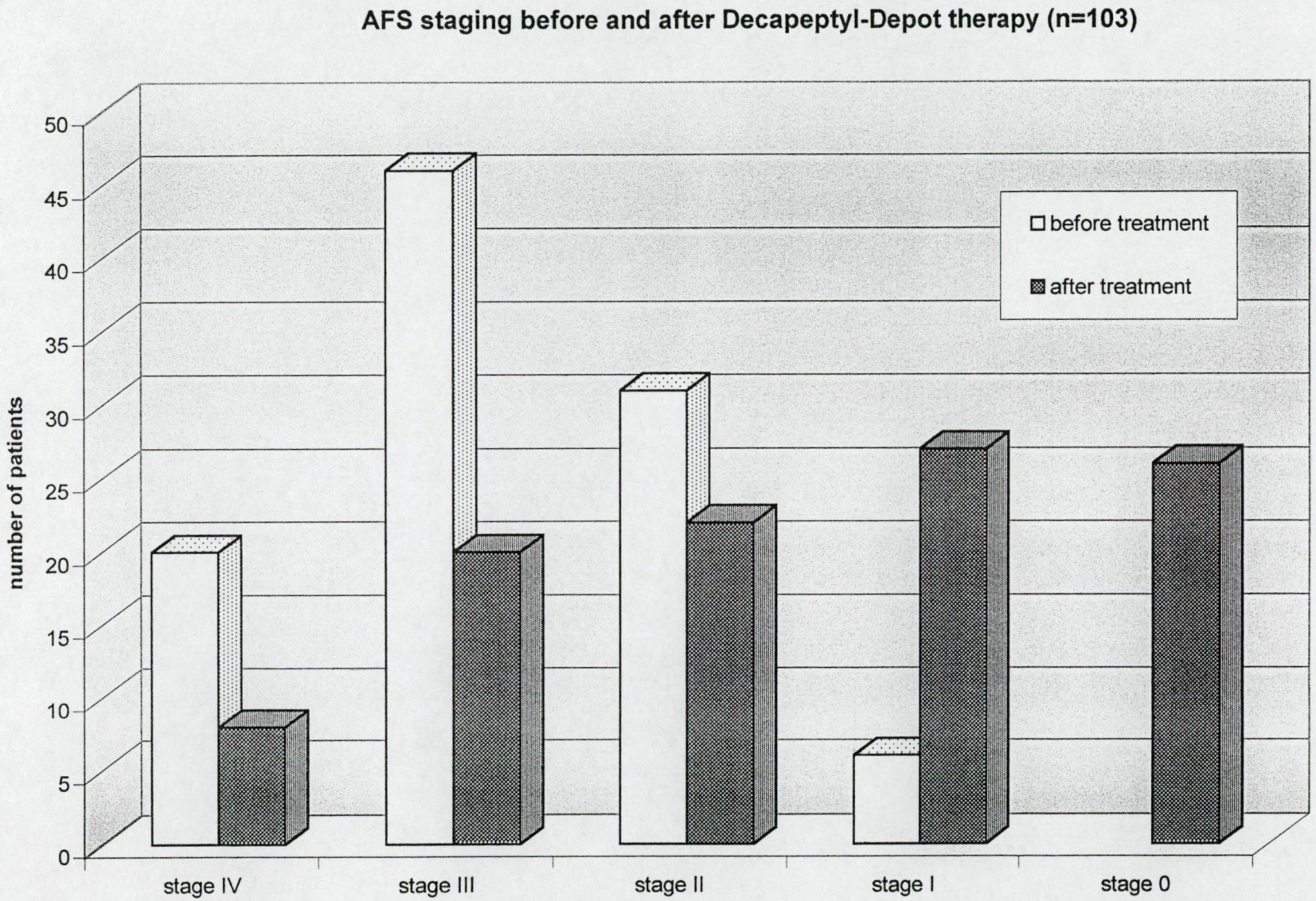
AFS staging before and after Decapeptyl-Depot therapy (n=103)
number of patients
50
45
40
35
30
25
20
15
10
5
0
before treatment
after treatment
stage IV
stage III
stage II
stage I
stage 0

Figure 8

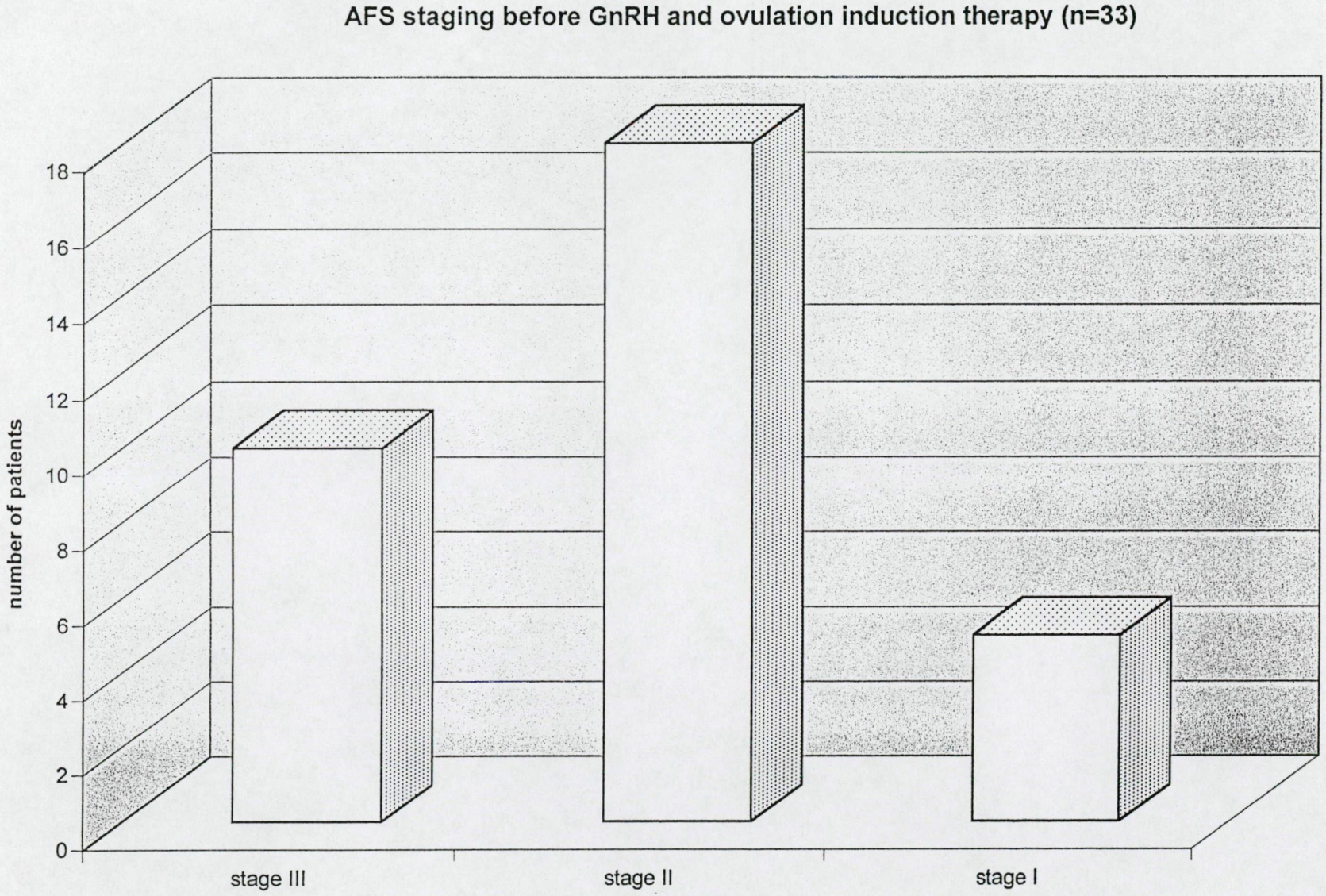
AFS staging before GnRH and ovulation induction therapy (n=33)
number of patients
0
2
4
6
8
10
12
14
16
18
stage III
stage II
stage I

Figure 9

IV. 4. Ovulation induction and BMI study

The BMI of the women involved in the study varied between 16 and 36. On this basis, they were divided into 4 groups: A: 16-19 (thin), B: 20-24 (normal weight), C: 25-27 (moderately overweight) and D: 28-36 (obese). Most of the women were in the normal BMI group. In the course of the superovulatory treatment, the induction was inefficient in 96 cases, and satisfactory follicle development did not occur. The risk of unsuccessful superovulatory treatment rise in a parallel with the shift from the ideal body weight (A: 1.5, C: 1.4, D :2.7) (Table XI)

Distribution of the patients according to the BMI and the relative risk of unsuccessful ovulation induction in the different BMI groups

BMI group	Number of cases (n=1144)	Unsuccessful ovulation induction (n=96)	Relative risk (%)	95% confidence interval
A: 16-19	178	18	1.5	0.8-2.5
B: 20-24	599	40	1	0.8-1.3
C: 25-27	286	23	1.4	0.9-2.1
D: 28-36	81	15	2.7	2.1-3.4

Table XI

As a result of DIUI carried out between 1992 and 1998, 412 pregnancies occurred, i.e. a success rate of 36%. Among the 412 pregnancies there, were 376 singlets, 29 twins, 6 triplets and 1 quintuplet. In the latter case, at the request of the couple, pregnancy reduction was performed in gestational week11, resulting in 2 live embryos. In spite of careful treatment and nursing, premature labour occurred in week 32 of the pregnancy. Caesarean section was

performed due to placenta praevia and live male twin foetuses weighing 1230 and 1390 g were delivered. From the pregnancies classified on the basis of the BMI, it can clearly be seen that DIUI can be applied most successfully in the case of a normal body weight (42%), whereas the pregnancy rate in overweight patients is only 21% (Table XII).

Distribution of the pregnancies obtained by artificial donor insemination in the different BMI groups

BMI group	Number of cases (n=1144)	Number of pregnancies	%
A: 16-19	178	50	28
B: 20-24	599	251	42
C: 25-27	286	92	33
D: 28-36	81	19	21

Table XII

V. Discussion

Endometriosis (where the endometrial islets, which may be located at sites other than the uterine cavity, change due to the hormonal cycle) is a benign disease; 95% of the cases occur in women of reproductive age. In the postmenopause, the disease is usually related to the administration of oestrogen and hormonal replacement therapy, but it can be found in men as well (46). However, the disease should be regarded as an aggressive change because of its frequent recurrence. The true incidence of the disease is not known, but its rate has increased in the last few decades. This may be only an apparent increase, due to the development of laparoscopy and the detailed description of the typical and atypical lesions. In cases where the diagnosis of endometriosis is uncertain, histological examination (with an optical or electron microscope) of the biopsy sample may help in clarifying the disease (6,29).

The underlying cause and the pathogenesis of the disease remain unclear, though several theories have been out forward in the last few years.

1. Transport through the uterotubal route is the most widely accepted idea for the spread of the disease (18). According to the implantation theory of Sampson, endometriosis may develop in the course of retrograde menstruation, when viable endometrial cells get access to the abdominal cavity, adhere to the peritoneum and bring about the changes (63). The latest clinical data suggest that the theory of retrograde menstruation can account for most cases of the disease. During diagnostic laparoscopy in the perimenstrual period a bloody peritoneal discharge can be found in more than 90% of women with an intact uterine tube.

2. According to Javert and to Scott et al., spreading through the lymphatic or blood vessels, respectively, also seems possible (30,66). Ridley et al. Demonstrated that the lesion can develop under experimental circumstances, as a result of an iatrogenic intervention, gynaecological operations including opening of the uterus, and Caesarean section (58).

3. The endometrium and the peritoneum develop from the same coelom epithelium. As suggested by Meyer and by Rosenfeld et al., the peritoneal mesothelium turns into endometrial tissue in the course of metaplastic transformation (49,60). The transformation can take place spontaneously, but it may be promoted by chronic irritation due to the retrograde menstrual fluid. Müller cell remnants can be placed in the tissues of the small pelvis while

Müller's duct is being developed, and in a few cases working endometrial gland and stroma can come into existence in response to the inducing effect of oestrogen.

4. The most recent findings show that a disturbance of the immune system also plays a role in endometriosis. In this female disease, an elevated humoral immune response and macrophage activity are present while decreases in cell-mediated immunity and in T cell and natural killer cell (NK) activity are also evident (15,26). It has been possible for some time to use a variety of methods to detect organ-specific autoantibodies directed against the endometrium (47).

5. Hereditary factors may also play a role in the disease. In the direct descendants of those suffering from this disease, the incidence is significantly higher than in the control group (28).

If black or dark-blue lesions are surrounded by star-shaped scars in the visceral or parietal peritoneum in the small pelvis, we describe the case as endometriosis, though many other macroscopic formations were also identified as endometriotic changes in the 1980s, due to the development of laparoscopy techniques. Biopsies involving different techniques contribute to the confirmation of the diagnosis when the peritoneal change is atypical in appearance. Nevertheless, the diagnosis is easy to make in full knowledge of the different symptoms and the peritoneal and ovarian changes.

V. 1. Nafarelin study

30 patients were involved in the study, with control laparoscopy in each case. The regular menstrual cycles discontinued in the second month of the treatment. The adhesions and the peritoneal lesions were taken into consideration. A tremendous improvement occured in the symptoms. Dyspareunia ceased in 22 cases (73%). During therapy with nasal nafarelin, Henzl et al. found that the dyspareunia was eliminated in 87%, whereas in the control group it was eliminated in 69% in response to oral danazol treatment (27,34,36). The pelvic pain lessened considerably or ceased in response to nafarelin, similarly as following treatment with danazol (23). Within 6 months following the treatment, 7 pregnancies occurred (23%), which was similar to the 28% found by Henzl et al. (27).

In general, one-third of patients with endometriosis have no symptoms, but the usual symptoms are pelvic pain and dyspareunia, mostly accompanied by infertility. The pain can be acute or chronic, one- or two-sided in appearance, and can radiate to the vagina, inguinal, gluteal and perianal regions, typically beginning 1-2 days before menstruation and lasting throughout the flow. The pain may be related to intestinal and bladder activity, if these organs are affected. The intensity of the pain and the feeling of discomfort are not related to the severity of the disease. Rather, it is the location, the depth of the endometriotic implants, and their relation to the adhesions in the small-pelvis and abdominal cavities that are the determining factors. The depth of infiltration of the disease in the uterosacral ligaments and the rectovaginal septum is positively associated with pelvic pain and dyspareunia. Many of these women also complain of pain during bowel motion while menstruating (dyschezia). Pain in the iliac fossae and flank may indicate involvement of the ureters, which may result in haematuria and dysuria. Rectal bleeding ocurs in about 20% of patients who have significant bowel endometriosis. Very occasionally, if an endometrioma is ruptured or bleeds, acute abdominal pain is the presenting symptom. The disease may be found in abdominal scars from previous surgery (e.g. a Caesarean section), causing superficial cyclic pain, tenderness and swelling.

Those in the age group 25-30 years generally suffer from small-pelvis endometriosis, while those in the age group 35-40 years suffer from the extragenital form. Extrapelvic endometriosis is estimated to occur in between 1% and 10% of patients with pelvic endometriosis. The disease has been reported to occur in almost all body structures, but the most common sites are the intestine, urinary tract, distal areas in the abdominal cavity, extrapelvic genital structures, lungs, skin and nervous system (31). Up to 50% of women with severe endometriosis have gastrointestinal forms, the most common sites being the rectosigmoid colon (50%), the appendix (15%), the small bowel (14%) and the coecum and colon (5%). The symptoms are diarrhoea, constipation, perimenstrual changes in bowel habits, rectal bleeding, pain on defecation, tenesmus, abdominal distension, small stools and colicky abdominal pain. Urinary tract endometriosis is thought to affect 1-4% of women with pelvic endometriosis; there have also been many cases without pre- or postoperative evidence of pelvic disease. Ureteral obstruction is associated with major morbidity; up to 30% of patients suffer a loss of kidney function, though renal endometriosis itself is extremely rare.

The bladder is the most common site of endometriosis in the urinary tract (80-90% of the cases), usually occuring in the trigone, the dorsal wall, at the uterovesicular juntion or transmurally. The ureters are involved in 10-15% of the cases, with the left being more commonly affected than the right. Urethral lesions are usually found in the distal third of the ureter below the pelvic brim. The symptoms are dysuria, superpubic pressure and back pain. The most common symptom of thoracic endometriosis is pneumothorax, followed by haemothorax, haemoptysis and asymptomatic lung nodule. Pleural lesions are more likely to cause pneumothorax or haemothorax. Haemoptysis is generally a result of lesions in the lung parenchyma. Diaphragmatic endometriosis may present as pain in the right upper quadrant or referred pain in the right shoulder. Endometriosis involving the skin is limited to the anterior abdominal wall at or below the umbilicus. Umbilical disease classically presents as a bluish tender mass, often associated with bleeding. The inguinal form presents as a painful mass. The overlying skin changes and cyclic symptoms vary. Endometriosis has been found in hernia sacs, in old inguinal scars and in the inguinal lymphatics. The most common site is the nerve in the pelvis. When sciatic pain occurs in relation to the menstrual cycle, it should suggest the presence of endometriosis. Involvement of the obturator nerve may produce pain and weakness in the proximal muscles of the thigh. Endometriosis has been reported in men undergoing treatment for prostate cancer by excision and orchidectomy and high-dose oestrogen therapy. The reduction in testosterone after removal of the testicles augmented by oestrogen therapy could account for these cases. For this reason, the pathological changes of other organs, together with the lesions of the internal reproductive organs, should be taken into consideration in the differential diagnosis of the disease (Table XIII).

Differential diagnosis of endometriosis

Abortion, complete
Abortion, incomplete
Abortion, inevitable
Abortion, missed
Abortion, septic
Abortion, threatened
Appendicitis, acute
Bowel obstruction, large
Bowel obstruction, small
Colitis, diverticulitis
Dysmenorrhea
Gastritis/peptic ulcer disease
Ovarian cysts
Ovarian, torsion
Pelvic inflammatory disease (PID)
Pregnancy, ectopic
Pregnancy, cervical
Urinary obstruction

Table XIII

V. 2. Nafarelin and triptorelin study

There was a noticeable decline in the symptoms of endometriosis. Dyspareunia was eliminated in 73% of the nafarelin group, as compared with 82% in the triptorelin group. Berquist et al. found that dyspareunia disappeared in 87% of the patients following triptorelin therapy, as compared with a 52% decrease in the control group, where a placebo was given (5,39). These results are similar to the findings of Gardo et al. who observed a clear reduction

or complete disappearance of the small-pelvic pain following nafarelin or triptorelin therapy together with danazol therapy (23). Similar results were obtained by Regidor et al., who reported a 20% pregnancy rate after buserelin acetate therapy. Seven (23%) patients in the nafarelin group became pregnant, as did 22 (21.4%) patients in the triptorelin group within 6 months following the treatment (57).

While it is easy to understand that advanced and severe endometriosis can cause disruption in the pelvis resulting in mechanical infertility, it is not similarly appreciated that minimal and mild endometriosis could have an impact on a woman conceiving and achieving a live birth. The link between endometriosis and infertility is based on the following observations:

- endometriosis is prevalent in patients presenting with infertility;
- the fecundity rate in women undergoing donor insemination is significantly reduced if they have endometriosis;
- the induction of experimental endometriosis in animals results in a decrease in fecundity rate.

There are a number of possible mechanisms that can lead to infertility in patients with mild-to-moderate endometriosis (Table XIV).

Possible mechanism of infertility in patients with mild-to-moderate endometriosis

Changes in peritoneal fluid

- **increase in volume**
- **presence of interleukins and tumour necrosis factor**
- **increased prostaglandin levels**
- **increased number of macrophages**

Ovulation disorders

- **anovulation**
- **hyperprolactinaemia**
- **abnormal follicular genesis**
- **premature follicular rupture**
- **luteal phase defect**
- **luteinized unruptured follicles**

Pelvic pain

Immunological abnormalities

- **T cells**
- **antigen-specific B cell activation**
- **anti-endometrial antibodies**
- **non-specific B cell activation**

Spontaneous abortion

Implantation

Table XIV

The mechanisms that may form the basis for an association between endometriosis and infertility remain controversial. In addition to the anatomic damage that may occur, severe endometriosis can cause significant adhesions, which can distort the architecture. This

interferes with both the release of the oocytes and their transfer into the Fallopian tubes. Several studies have shown an increase in the volume of peritoneal fluid in women with pelvic endometriosis, but the correlation between fluid volume and fertility has not been consistent (1,2,74). Interleukins and tumour necrosis factor in the peritoneal fluid of endometriosis patients are involved in the inhibition of sperm motility and function, oocyte fertilization and embryo growth (17,70). The peritoneal fluid from women with endometriosis has a negative impact on the sperm motility, and the sperm binding to the zona pellucida has been shown to be reduced in vitro. Several groups have reported increased prostaglandin levels in the peritoneal fluid in endometriosis (1,74). Prostaglandins alter the tube motility and the collection of oocytes and can lead to luteinized unruptured follicle syndrome and corpus luteum defects or luteolysis. The number of macrophages in the peritonel fluid is increased in women with pelvic endometriosis. This is associated with the increased production of free radicals, which reduces the sperm motility (2). These macrophages are thought to be more activated, thereby affecting the rate of sperm phagocytosis and producing factors that might make the peritoneal fluid less hospitable to oocytes and sperm (50,51,73). Anovulation has been reported in 17-27% of patients with endometriosis, while others observed an increased occurrence of hyperprolactinaemia and effects within the peritoneal environment (68,71). A luteal phase defect, detected by out-of-phase endometrial development or asynchronous development of the endometrial glands and stroma, could be a consequence of abnormal follicular development, inadequate production of progesterone or a lack of response of the endometrium to progesterone (7). Luteinized unruptured syndrome has been described in monkeys with surgically induced periovarian endometriosis (64). Theoretically, a decrease in the number of LH receptors could be the underlying mechanism, but ultrasonographic studies have failed to show a consistent increase in incidence of this syndrome in patients with endometriosis. Pelvic pain can lead to a reduction in the frequency of intercourse and therefore reduce the likelihood of pregnancy. As endometriosis involves the transplantation of autologous endometrium and T cells are concerned with the rejection of homografts, changes in T cell function have long been suspected in endometriosis. Controversy exists, however, regarding the pattern of circulating leukocytes, as some, but not all, investigators have reported decreased numbers of lymphocytes. To add confusion, increased numbers of T cells and B cells, and higher CD4:CD8 ratios in the blood and peritoneal fluid have also been

reported in women with endometriosis. Some researchers suggest that a subset of T cells is functionally deficient in women with endometriosis. Recently, a specific deficiency in lymphocyte-mediated cytotoxicity towards autologous endometrial cells was observed and was thought to be a result of an NK cell dysfunction. A lowered cytotoxic effect has been confirmed by other investigators, but not an NK cell dysfunction (52). A definitive mechanism by which altered T-cell function could cause infertility has not yet emerged. Anti-endometrial antibodies directed towards endometrial cell antigens have been reported in the serum of women with endometriosis, but why they exist is controversial. Anti-endometrial antibodies have also been detected in women with a wide range of pelvic pathology (47,69,72). Non-specific B-cell activation, an autoimmune syndrome characterized by polyclonal B cell activation, has been suggested as a cause of infertility, thought the exact mechanism is unclear (3). An increased incidence of spontaneous abortion has been reported in patients with endometriosis, with a corresponding decrease after the endometriosis has been treated. Abnormalities in prostaglandin function could be the possible mechanism (54). In a non-randomized study of patients with endometriosis, two-thirds of whom had suffered a previous miscarriage, the pregnancy rate was similar whether these patients had had corrective surgery or not, but the miscarriage rate was higher in the group that had not been treated (55). The integrins are a group of compounds essential for cell adhesion. A deficiency of integrin av 3, a component of the embryo implantation cascade in the uterus, leads to a possible reduction in embryo implantation in women with early-stage endometriosis. However, this appears to be correctable when the disease is treated (59).

V. 3. Triptorelin and ovulation induction study

During the 6-month treatment with analogues, certain side-effects due to hypo-oestrogenism emerged some 2-3 weeks after the first triptorelin injection. The menopausal symptoms gradually increased up to months 4 and 5 of the treatment, and a slight reduction was noticed in month 6. The ovulation induction therapy was started according to the monofollicular protocol, on day 21 of the cycle after the last analogue injection was given. In the first month of the induction treatment, 5-8 (6.8 on average) ova reached a size of 20 mm. This number is significantly different from the numbers of mature follicles (3.9 and 4.2)

obtained after long-term therapy with GnRH analogue and hMG by Schmutzler and Leyendecker, and slightly lower than the results of Frydman, who obtained on average 8.4 ova after the long-term protocol (21,37,45,65). Hyperstimulation was not observed during the trial, so luteinization was performed in each case. After homologue artificial therapy, 15 (45%) patients became pregnant, 3 of these cases involving twins. This compares to efficiency rates of 25% and 27% following embryo transfer obtained by Leyendecker and Frydman (21,35,45), respectively. The 45% success rate is probably mostly due to the young age of the patients, the rebound effect following inhibition and the fact that no other causes of infertility apart from endometriosis were observed. This may be compared with the report by Koloszár, whose success rate was 36% following donor insemination, but in this study the patients' body weight was not ideal. When the patients' weight was normal, the success rate was similar (42%), but in the low and high body weight groups the rate was lower (28% and 21%) (42). To sum up, the above course of treatment (6-month GnRH analogue therapy, the ovulation induction following the monofollicular protocol and HIUI) can be applied successfully in the treatment of infertility caused by endometriosis.

The findings of the physical examination tell us about the extent of the disease. Usually, non-specific small-pelvic tenderness can be found in an examination. The tenderness is localized to the adnexal region if the ovaries are affected, while in the case of anaffected peritoneum the sacrouterine ligament can be painful and sensitive, or the tenderness is localized to the Douglas pouch. In the case of a "frozen pelvis", the uterus is in a fixed retroposition due to the extended adhesions and is accompanied by severe small-pelvic pain. If the ovarian endometriosis ruptures, acute abdomen may develop. However, if the gastrointestinal tract becomes affected due to the extended adhesions, obstructions can occur. Cyclic haemoptoea and pneumothorax can develop in extra-abdominal diseases (31).

Urine analysis, a pregnancy test and, if required, evaluation of the serum β-HCG level and examination of a vaginal smear by electron microscopy may be of great assistance in the differential diagnosis, together with a detailed medical history and bimanual examination. A TUS examination may be useful in the assessment of the lesion in the small pelvis. The reliability of TUS depends on the nature of the lesions. In the detection of endometriomas, TUS is reported to have a sensitivity of 80% and a specificity of 95%. In contrast, the sensitivity of TUS in the detection of focal implants is poor and may be as low as 10%.

Typically, endometriomas are visualized as predominantly cystic masses with thick walls, often with diffuse acoustic enhancement or scattered internal echoes and may contain septae, dependent echoes or fluid levels. Computerized tomography and magnetic resonance imaging (MRI) are more sensitive in detecting small-pelvic lesions than an ultrasonographic scan, but their uses are limited in the case of multiple small-pelvic endometriosis. MRI detects endometriomas, ovarian adhesions and extraperitoneal masses. It may be useful for revealing changes in the size and number of endometriotic lesions during therapy, for detecting the invasion of the nerves, as in sciatic endometriosis, and for identifying abdominal wall lesions. MRI findings do not correlate with the stage of the disease. Taking unadulterated (native) abdominal X-ray pictures is not recommended until other diagnostic procedures suggest that there is an intestinal obstruction. Three serum immunoassays have been tried in the diagnosis of endometriosis: CA-125, placental protein 14 (PP14) and antibodies to endometrium. Of these, CA-125 shows the most promise. CA-125 is an ovarian epithelial tumour antigen that is detected by a monoclonal antibody designated OC-125. The incidence of elevated CA-125 levels increases with the severity of the disease, and the mean concentrations clearly correlate with the disease stage. However, since the CA-125 levels are not elevated in mild forms of endometriosis, and are elevated during and immediately after menstruation, CA-125 is not useful as a screening test. It could, however, be valuable in monitoring the effects of therapy. CA-125 measurement has been successfully used in conjunction with other examinations (e.g. TUS) (19,53,73).

Hormonal therapy has been the main medical treatment for endometriosis for half a century. In the 1940s and 1950s, diethylstilboestrol and methyltestosterone were used, but these therapies in the treatment of the disease should now be given only a historical mention, as their use is no longer accepted, due to the frequent occurrence of side-effects. Kistner, in a paper published in 1958, reported on pseudopregnancy, which generally developed after high-dose oestrogenic-progestogenic therapy. He achieved significant results in reducing the medical complaints (41). After the use of antigonadotropin therapy by Greenblatt in 1971, it was applied as a standard drug in measuring the effectiveness of the treatment of endometriosis (24). Danazol is a synthetic by-product of testosterone, with a half-life of 4.5 hours. Peak levels are reached 2 hours after oral ingestion and it is undetectable after 8 hours. It is metabolized in the liver, and the principle metabolite, methylethisterone, exhibits mild

progestogenic and androgenic activity. Danazol has a direct effect on steroidogenesis, acting on cholesterol cleavage enzymes and on intracellular steroid receptors. It has an indirect action by decreasing the GnRH pulse frequency, which may suppress ovulation. The most common side-effects are related to the hyperandrogenic state: weight gain, oily skin and hair, nausea, acne, muscle cramps and hirsutism. Deepening of the voice, though uncommon, is irreversible. Hypo-oestrogenic side-effects, such as hot flushes, decreased breast size and reduced libido, may also occur. Danazol has multiple metabolic side-effects, the most important of which relate to the blood cholesterol. It decreases HDL and increases LDL levels, which must be taken into account as the drug is given for long periods (6-9 months). Its use should be avoided in women with a history of liver disease. Gestrinone, a progesterone agonist/antagonist, which is a 19-nortestosterone derivative, was used primarily as a long-term contraceptive drug (12). The actions of gestrinone result in amenorrhoea and endometrial atrophy, similar to other androgen steroid analogues (e.g. danazol). It acts both centrally and peripherally to reduce oestradiol and obliterate the midcycle LH surge. It has a long half-life, allowing oral administration 2-3 times weekly. Gestrinone has fewer androgenic symptomatic and metabolic effects as compared with danazol, and fewer hypo-oestrogenic side-effects. Operative castration and subsequent hypoestrogenism would be the real solution in the treatment of the disease, but this is out of the question with young women of reproductive age. The development of GnRH analogues which reversibly inhibit the function of the ovary in a hormonal way, was a breakthrough in the treatment of the disease (14,50). GnRH is a decapeptide produced by the arcuate nucleus of the hypothalamus and is secreted episodically into the pituitary circulation to regulate the release of LH and FSH. GnRH can be metabolized by pituitary endopeptidases (cleavage at positions 5-6 and 6-7) and carboxyamide peptidase (cleavage at position 9-10). These degradative steps inactivate the molecule and are responsible for the short, 2- to 8-minute half-life of GnRH. Nafarelin acetate and triptorelin are analogues of GnRH in which the amino acid sequence is ranged. This results in GnRH analogues with high affinity for the GnRH receptor and a very long half-life (240 minutes), because the synthetic derivative is more resistant to the effect of endopeptidase. Because of the long half-life and enhanced potency of the GnRH analogues nafarelin and triptorelin, administration of these compounds results in prolonged and continuous occupancy of the pituitary GnRH receptor. The effects of nafarelin and triptorelin administration are similar to

those of a continuous high-dose infusion of GnRH. The analogue administration results in an initial phase in which there is release of LH and FSH. This is followed by a protracted state of decreased gonadotropin secretion. The second phase of this action of the analogues ultimately leads to a hypogonadal state, with a marked decrease in ovarian oestrogen production. The mechanism by which nafarelin and triptorelin produce an initial agonist effect (initial release of LH and FSH), and then a paradoxical and marked decrease in LH and FSH secretion, is not known at the molecular level. Two proposed mechanisms are desensitization and down-regulation. Desensitization refers to an uncoupling of the activated GnRH receptor from the intracellular mediators of signal transduction. Down-regulation refers to a decreased number of unoccupied cell-surface GnRH receptors. In contrast with danazol, the only protein with which nafarelin and triptorelin interact is the pituitary GnRH receptor. The decreases in pituitary LH and FSH secretion produced by these analogues result in decreases in ovarian oestradiol and progesterone production. Virtually all of the effects of the analogues are caused by the decreased ovarian oestradiol and progesterone production. As noted previously, agonists truly display "molecular specificity", interacting with the pituitary GnRH receptor and few other proteins. In contrast, danazol interacts with at least a dozen proteins, including enzymes of steroidogenesis, multiple classes of steroid receptors, and circulating steroid-binding proteins. The endocrine pharmacology of agonists are the best understood by comparing them with danazol. Nonetheless, the clinical profiles of women taking danazol appear to be quite different from those of women taking the GnRH agonist. Specifically, patients treated with danazol tend to have a greater degree of weight gain and bloating than have women treated with agonists. Further, women treated with danazol have a higher incidence of deepening of the voice, acne, oily skin and unwanted growth of facial hair. In contrast, the GnRH agonists tend to be associated with a higher incidence of hot flushes, vaginal dryness and decreased libido. The effects of the hormones employed in the treatment of endometriosis are listed in Table XV.

Endocrine effects of hormones used in the treatment of endometriosis

	Gonadotropic secretion	Function of the ovary	Endometrium	Other effects
Pseudopregnancy (oestrogen/progestogen)	**inhibition**	**inhibition**	**decidual**	**oestrogenic, progestogenic**
Progestogen	**variable inhibition**	**variable inhibition**	**decidual**	**progestogenic**
Pseudomenopause (danazol)	**inhibition**	**inhibition**	**atrophy**	**androgenic, anabolic, immunological**
GnRH agonists	**short stimulation, then inhibition**	**short stimulation, then inhibition**	**atrophy**	**menopausal symptoms**

Table XV

V. 4. Ovulation induction and BMI study

It is well known that ovulatory disorders are more frequent in lower and higher body weight groups. Furthermore, the efficiency of ovulation induction with clomiphen citrate can be considerably enhanced of decreasing a pathologically high body weight, or increasing a too low weight, towards the ideal condition. The ideal body weight can ensure a favourable interior environment for the function of the endocrine system; additionally, a pathological body weight may play a role in luteal failure (40). In order to maintain the ideal body weight, the intact functioning of certain areas of the hypothalamus is necessary. It has been found in animal experiments that, following lesions of the medial preoptic part of the hypothalamus,

the body weight increases and an ovarian malfunction occurs. Furthermore, in female animals, oestrogen withdrawal by castration leads to an increased food intake. The inhibitory effect of oestrogen on nutrition takes place through the neuropeptide-Y and the galanin peptidergic system (22). When a total of 1144 infertile women were treated by DIUI, unsuccessful ovulation induction was found in 96 cases. The obese women (BMI: 28-36) had a relative risk of unsuccessful ovulation induction of 2.7 (95% CI = 2.1-3.4) as compared with women with a lower or normal body weight (BMI: 20-24). The effect was smaller in women with a BMI of 25-27 or <19 (RR = 1.4, 95% CI = 0.9-2.1 and RR = 1.5, 95% CI = 0.8-2.5, respectively). During DIUI treatment, 412 pregnancies occurred. The pregnancy rate achieved by insemination was 28% (50 pregnancies in 178 cases, BMI 16-19), 42% (251/599, BMI 20-24), 33% (98/286, BMI 25-27) and 21% (19/81, BMI 28-36), in the different BMI groups.

VI. Conclusions

Nafarelin and triptorelin are synthetic GnRH analogues which are 200 times as effective as the endogenous protein. They stimulate the release of LH and FSH from the anterior lobe of the pituitary, but this effect shows a gradual decrease in continuous administration as a result of desensitization of the pituitary due to the down-regulation of the receptors. The treatment aims at the pharmacological manipulation and changing of the milieu in the small pelvis. In response to continuous administration, nafarelin and triptorelin inhibit the pituitary - ovary axis and the evoked hypooestrogenic condition proves to be very efficient in the therapy of the endometriosis of the small pelvis, with rapid decreases pain and other symptoms. The induced oestrogen deficiency condition is tolerated by the patients quite satisfactorily.

In conclusion, we can state that nafarelin and triptorelin can be successfully applied in the treatment of endometriosis and its symptoms (dysmenorrhoea, dyspareunia and infertility). Besides having the advantage of a practical depot formulation which ensures a sustained and continuing release of the analogue, triptorelin provides a very good suppression of gonadal steroidogenesis. We found that the GnRH analogue therapy decreases the serum FSH and LH concentrations; the oestradiol levels in all patients, were initially in the postmenopausal range, were all within the normal range after the therapy. In fact, although this therapy is often referred to in the literature as "medical castration", it has been hypothesized that complete inhibition of gonadal steroidogenesis is rarely achieved with the doses of agonists in current use. In the trials, significant side-effects other than symptoms of an oestrogen deficiency (which seem to be acceptable and which promptly disappeared after the suspension of agonist therapy) were not observed either during the treatment or in the follow-up period. A cyclic pituitary-ovarian function returned within 2 months of the end of therapy. In no patient did the trial end before the set period due to side-effects or unexpected complications. The patients overcame the reversible hypoestrogenism quite well.

Results of the investigation

1. We sought to explain the incidence of endometriosis due to infertility in our region, the clinical application and efficiency of nafarelin in cases of endometriosis diagnosed during laparoscopy, and the decline in activity and expansion of the disease in the course of the 6-month therapy. We performed 122 laparoscopies in the investigation of infertility and found endometriosis to be the underlying cause in 30 patients (25%). After treatment of the adhesions and the peritoneal lesions was taken into consideration, there was a marked improvement in the symptoms.

2. We compared the clinical applicability, the efficacy, and the usual and unexpected side-effects of two GnRH analogues, nafarelin and triptorelin, and the reduction in the activity and spread of the disease after the 6-month course of treatment. 133 patients with laparoscopically-diagnosed endometriosis were treated with the GnRH analogues. No significant differences were observed between the groups in the frequency of hot flushes. The hot flushes disappeared completely from the second month on following the therapy. In the first month after the start of the medical therapy, symptoms characteristic of the menopause (colpoxerosis, nervousness, mood swings, a reduction in libido and a decrease in the size of the breasts, accompanied by hot flushes) appeared due to hypoestrogenism. As regards side-effects, no significant difference was found between the two groups. There was no significant difference between the two groups in the serum hormone levels during and after the analogue therapy. We found no significant complications with either therapy formulation. There was a noticeable decline in the symptoms of endometriosis. Dyspareunia vanished in 73% of the nafarelin group, as compared with 82% in the triptorelin group.

3.1. We examined the efficiency of HIUI following ovulation induction. This was performed, after triptorelin therapy, in patients where laparoscopically diagnosed endometriosis had elucidated the cause of their infertility and pelvic pain. At our Department, ovulation induction and HIUI were performed after GnRH analogue therapy in 33 patients with laparoscopically diagnosed endometriosis. The intervention resulted in pregnancy in 15

(45%) cases, of which 3 were twin pregnancies. In 1 case, a spontaneous abortion took place in week 15 of the pregnancy.

3.2. We sought a correlation between the female body weight and the efficiency of DIUI, with special regard to successful superovulatory treatment and ensuing pregnancies. At our Infertility Outpatient Unit, 1144 married couples attending between 1992 and 1998 asked for DIUI treatment. As a result of DIUI between 1992 and 1998, 412 pregnancies occurred, which means a success rate of 36%. Among the 412 pregnancies, there were 376 singlets, 29 twins, 6 triplets and 1 quintuplet. When the pregnancies were classified on the basis of the BMI, it could be seen clearly that DIUI can be applied most successfully in cases with a normal body weight (42%), while the pregnancy rate in overweight patients is only 21%.

In the first month of induction treatment, 5-8 (6.8 on average) ova reached a size of 20 mm in both groups. Hyperstimulation was not observed during the trial, so luteinization was performed in each case where the ovulation induction was successful. We established that there was no significant difference in pregnancy rate between the two IUI-treated groups, who had or who were free of endometriosis, but the body weight was in the normal range. After GnRH analogue treatment, ovulation induction and IUI therapy, 15 (45%) patients became pregnant as compared with ovulation induction and IUI treatment, where the success rate was 36%, but in this study the patients body weight was not ideal. When the patient's weight was normal, the success rate was similar (42%), whereas in the low and high body weight groups the rate was lower (28-21%).

4. We suggest a treatment protocol for infertile patients who have endometriosis that calls for remove al of the endometriomas during laparoscopy, and decrease of the number of adhesions and endometrial plaques; the patients have to start ovulation induction as soon as possible, after the analogue therapy, and to supplement it with other assisted reproductive technology if required, because the success rate was significantly lower (21.8%) if we did not use ovulation induction management after the analogue therapy. We conclude that the analogue therapy and the continuous ovulation induction with monofollicular protocol and IUI represent a good therapeutic option in the management of infertility due to endometriosis. We found that 85% of the pregnancies occurred in the first two cycles.

To sum up, the above course of treatment (6-month GnRH analogue therapy, and ovulation induction following the monofollicular protocol and IUI) can be applied successfully in the treatment of infertility caused by endometriosis. Further efforts must be made to achieve and maintain the ideal body weight, since in this way the efficiency of ovulation induction and IUI can be enhanced.

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VIII. Acknowledgements

I am greatly indebted to my tutor, Professor Attila Pál, M.D., Ph.D. for his help.

I wish to express my grateful thanks to József Daru, M.D., Ph.D. for his professional guidance.

I am deeply indebted to Professor László Kovács M.D., D.Sc. for him support.

My thanks are due to colleagues at the Department of Obstetrics and Gynaecology, Albert Szent-Györgyi Medical and Pharmaceutical Center University of Szeged, Szeged, Hungary.

I am especially grateful to my family for their patience and support.